CANADA/U.S. SPRUCE BUDWORMS PROGRAM - West

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Title: ISOZYME CHARACTERISTICS AND GENETIC DISTANCES BETWEEN SPRUCE BUDWORM SPECIES

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1. SUMMARY

The genetic makeup of populations of 5 Choristoneura species was compared using starch gel electrophoresis. Species include C. occidentalis Freeman from Idaho, C. biennis Freeman from British Columbia, C. retiniana (Walsingham) (= viridis Freeman) from Oregon, C. lambertiana ponderosana Obraztsov from Colorado, and C. fumiferana (Clemens) from Maine. When variation at individual gene loci was examined, intraspecific variation was often as great, and sometimes greater, than interspecific variation and few significant differences were noted among the species. The highest levels of overall genetic similarity occurred among C. occidentalis, C. biennis, and C. retiniana. Relatively greater genetic distances were found between this group and C. lambertiana and C. fumiferana. C. fumiferana was most distantly related to all other groups. Genetic identity values fell well within the range more commonly associated with conspecific populations rather than with separate species. Data therefore suggest that reproductive isolation among these species is not well established.

2. INTRODUCTION

The spruce budworm complex, initially considered a single species, Choristoneura fumiferana, was revised in 1967 with descriptions of several new species (Freeman 1967, Freeman and Stehr 1967). The spruce/fir-feeding "Fumiferana complex" presently includes the eastern species, C. fumiferana (Clemens), and 4 western species: C. occidentalis Freeman, C. retiniana (Walsingham) (= viridis Freeman), C. orae Freeman, and C. biennis Freeman. The pine-feeding "Lambertiana complex" consists of the eastern C. pinus Freeman (with 2 subspecies) and the western C. lambertiana (Busck) (with 3 subspecies). A third group, the "Carnana complex", is believed to be associated with Douglas-fir in California and is still of uncertain affinity (Powell 1980).

Damage to North American forests caused by members of this group is extensive. During the past several decades, the area infested by western spruce budworm, C. occidentalis, in the western U.S. and British Columbia and, to a lesser extent, the Modoc budworm, C. retiniana, has fluctuated between 1.6-2.6 million hectares (4-6.5 million acres) (USDA Forest Service 1979a). The eastern spruce budworm, C. fumiferana, has invaded approximately 60 million hectares (150 million acres) of spruce/fir forest in the eastern U.S. with timber losses as high as 282 million cubic meters (10 billion cubic feet) (USDA Forest Service 1979b).

The ongoing Canada/U.S. Spruce Budworms Program aims to provide effective, safe, and economical controls for C. fumiferana and C. occidentalis and to develop management strategies for budworm susceptible forests. A basic premise of this large-scale integrated research effort is that the two budworm species can be identified and that their relationships to other members of the genus are known. However, disagreement still exists as to the number of Choristoneura species and subspecies, their levels of taxonomic distinctness, their geographic distributions, and the names applied to them.

According to Powell (1980), the principal source of confusion among western Choristoneura groups lies in the complex variation among populations. In sympatric populations, the degree of reproductive isolation is essentially unknown and allopatric populations show much variation in size and color of adults, larvae, and pupae, and in host plant preferences. Both polymorphic and continuously variable features occur and characters conventionally used by taxonomists to define species of Lepidoptera (e.g., genitalic structures) are not sufficiently differentiated in this group to provide reliable morphological indicators of genetic and behavioral compatibility among populations.

Recent developments in molecular genetics offer useful techniques for resolving systematic problems of this type. Of the various methods for determining levels of genetic differentiation among taxa, with respect to the degree of sensitivity and ease of analysis, gel electrophoresis is ideal for comparing subspecies, species, and closely related genera (Avise 1974, Bush and Kitto 1978, Berlocher 1979, Gonzalez et al. 1979). Electrophoretic techniques are particularly good for determining if reproductive isolation occurs between species. Because there is usually a one-to-one relationship between an individual's electrophoretic phenotype and its genotype, the capability of this method to detect variation at single gene loci removes ambiguity inherent in other, more traditional, approaches. Electrophoresis also permits comparison of gene frequencies between populations at a large number of loci, a direct though conservative estimation of genetic differentiation, and often provides insights that extend beyond those obtained by more traditional criteria.

In the study reported here, the genetic makeup of populations of 5 Choristoneura species was compared using starch gel electrophoresis. Our aim was to estimate levels of reproductive isolation among the species. In addition to specimens of the western spruce budworm (C. occidentalis) and the eastern

spruce budworm (C. fumiferana) we also examined specimens of C. biennis, C. retiniana, and C. lambertiana ponderosana OBraztsov. A summary of the distinguishing features of these species is given by Freeman and Stehr (1967) and a distribution map is shown in Fig. 1. Of these 5 species, the first two are the most widespread and most destructive. C. biennis, the two-year budworm, occurs at high elevations on alpine fir and Engelmann spruce in SE British Columbia and SW Alberta, areas where C. occidentalis is found at low elevations on Douglas-fir (Shepherd and Dolph 1979). Host preference, dark coloration of larvae and pupae, and the occurrence of a 2nd diapause in the 4th instar in C. biennis distinguish the two species. The Modoc budworm, C. retiniana, has been reported predominantly on white fir in south-central and in NE California although phenotypically similar fir-feeding populations occur along the length of the Sierra Nevada, Tehachapi, and Transverse Ranges in California (Powell 1980). The primary distinguishing feature between C. retiniana and C. occidentalis is the distinct green hemolymph coloration of C. retiniana which is readily seen through the unpigmented cuticle of larvae and pupae. Sanders (1971) found differences between the sex pheromone of C. retiniana and that of C. fumiferana, C. occidentalis, and C. biennis. The latter 3 species share a very similar sex pheromone.

C. lambertiana ponderosana is found in Colorado, Wyoming, and Montana. It is distinguished from other western species primarily by feeding habit; its host is ponderosa pine. It is also distinctive in having forewings more diffusely marked and lacking rust or reddish markings compared to other subspecies of C. lambertiana (Stevens et al. 1977, Powell 1980).

2b. OBJECTIVES

The objectives of this study, as outlined in the proposal submitted 5 February 1979 and funded in June 1979, were to:

1) Obtain samples of *Choristoneura occidentalis*, *viridis* (=*retiniana*), *biennis*, *lambertiana*, and *fumiferana* from field locations throughout North America.
2) Examine isozyme variation within and among groups using electrophoresis.
3) Estimate species relationships (degrees of genetic similarity or distance) and identify those isozyme patterns which can be used to distinguish the species and as markers in studies of SBW distribution and dispersal.
4) Provide results to other CANUSA researchers in a cooperative effort to clarify systematic relationships among spruce budworm species.

3. METHODS

Samples of later-instar larvae were obtained from the following sites:

1) C. occidentalis -- 300+ larvae from Payett National Forest, Valley County, Idaho, on Douglas-fir and grand fir.
2) C. biennis -- 600+ from Cariboo Mts, SE British Columbia, on subalpine fir.
3) C. retiniana -- ca. 50 larvae from Fremont National Forest, Lake County, Oregon, on white fir.
4) C. lambertiana ponderosana -- ca. 90 from Lyons, Boulder County, Colorado, on ponderosa pine.
5) C. fumiferana -- 200+ from Washington County, Maine, on balsam fir.

Techniques used for electrophoresis of spruce budworms are described by Willhite (1979). Isozyme banding patterns were converted to genotype and allele (allozyme) frequencies for quantitative comparison of species. The genetic makeup of each species for each locus was compared to expected Hardy-Weinberg values with a chi-square test. Contingency tests, based on the observed number of each allele present, were used to detect differences in gene frequencies at single loci. Loci differing significantly between species with respect to the distribution of particular gene types were considered for use as markers or distinguishing features of species. Data

from all loci were used to calculate Nei's (1972) genetic identity and genetic distance values. Genetic identity values vary from zero to one where one indicates total genetic similarity. Nei's genetic distance is interpreted as a measure of the average number of electrophoretically detectable allelic substitutions per locus which have accumulated since two populations or species separated from a common ancestor. These values are useful estimates of genetic relationships when used at the species and population levels (Ayala 1975). Using the genetic identity values, a dendrogram was constructed, using the complete linkage clustering method (Sneath and Sokal 1973), to aid visualization of genetic relationships among species.

Results were compared to those reported for Choristoneura species by Stock and Robertson (1980) and for C. occidentalis in Idaho and Montana by Willhite and Stock (1980).

4. RESULTS

Data were obtained on 18 gene loci representing 12 enzyme systems in the 5 Choristoneura species (Table 1, Fig. 2). Fourteen of the 18 loci (AAT1; CK; EST1, 2, 3, 5, and 6; IDH; LDH; LAP2 and 3; ME; MDH2; PGI) were polymorphic in at least one species, with esterases being most highly polymorphic. AAT2, AGPDH, 6GPDH, and XDH were monomorphic in all groups.

When variation at individual loci was compared among species (data reported here) and among samples of a single species, C. occidentalis (Willhite 1979), intraspecific variation was often as great, and sometimes greater, than interspecific variation. However, some significant differences among the Choristoneura species were noted. At AAT1 and EST5, C. fumiferana

was distinguished by a different common allozyme from the other species. At LAP2, C. fumiferana and C. retiniana both had a different common allozyme than the other species. At all 3 of these loci, C. occidentalis and C. biennis were most similar.

At EST2, C. retiniana had a lower frequency of the common allozyme than other species. However, the range of variation in frequency of the common EST2 allozyme (.66-.96) reported among C. occidentalis samples (Willhite 1979) came close, at its lower limit, to the frequency of the common allozyme recorded for C. retiniana in this study (.58). At LAP3, C. retiniana was more polymorphic than other groups. C. lambertiana had a higher frequency of EST6 allozyme 2 than the other species and a different common allozyme at EST1. Again, however, the range of C. occidentalis intraspecific variation at these loci encompassed these differences. C. lambertiana was the only species polymorphic at MDH2. At EST3, C. lambertiana had a lower frequency of the common allozyme than the other species, but high levels of intraspecific variation noted at this locus among C. occidentalis samples suggest that this is not a significant distinguishing feature between C. occidentalis and C. lambertiana. Over all loci, no significant differences in gene frequency were seen between C. occidentalis and C. biennis.

Greater insight into species relationships was obtained when data from all loci were compared simultaneously. Genetic identity values (Table 2) ranged from .99 (between C. occidentalis and C. biennis) to .84 (between C. fumiferana and C. lambertiana). Genetic distances (Table 2) showed that, between C. occidentalis and C. biennis, only an estimated 0.7 electrophoretically detectable allelic substitutions for every $\frac{100}{\wedge}$ loci have occurred since the divergence of these two groups from a common ancestral population. Between C.

fumiferana and C. lambertiana, an estimated 17 allelic substitutions for every 100 loci have occurred since their divergence from a common ancestor. C. retiniana was most closely related to C. occidentalis/biennis and, of the 4 western species, C. lambertiana was most distantly related (Fig. 3).

Differences among species noted in this study are consistent, in most cases, with differences noted among species by Stock and Robertson (1980). However, C. retiniana (= viridis) from the Berkeley laboratory colony was significantly different from field-collected C. retiniana at both EST2 and 3. Thus, results of the earlier study suggested a higher level of genetic divergence between C. occidentalis and C. retiniana than is the case when field populations are compared. Also, an appreciably higher frequency of silent alleles at EST2 was found in the 1977-collected C. occidentalis from McCall, Idaho (Stock and Robertson 1980). In neither the 1978 (Willhite 1979) or 1979 (reported here) McCall collections of C. occidentalis were silent alleles detected at the EST2 locus. This difference could reflect a temporal shift in frequencies at this locus, since recent studies have shown that frequencies of the EST2 silent allele are highly labile[1].

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[1] Stock, M. W., and J. L. Robertson. Effect of insecticides on esterase isozymes of western spruce budworm, Choristoneura occidentalis Freeman (Lepidoptera:Tortricidae), during larval development. In preparation.

Table 1. Isozyme frequencies in samples of 5 Choristoneura species.

Enzyme	Abbrev.	Locus	Allozyme	Relative Mobility	Species: occidentalis	retiniana	fumiferana	biennis	lambertiana
Aspartate aminotransferase	AAT	1	1	.46	0	0	0	0	0
			2	.42	0	0	.68	0	0
			3	.32	0	0	.25	.04	.02
			4	.22	1.0	1.0	.07	.94	.95
			5	.12	0	0	0	.02	.03
		2	1	.10	1.0	1.0	1.0	1.0	1.0
Creatine kinase	CK	1	1	.36	.01	0	0	.01	0
			2	.28	.97	1.0	.96	.98	1.0
			3	.20	.02	0	.04	.01	0
Esterase	EST	1	1	.88	0	.08	0	0	0
			2	.85	.69	.59	.56	.57	.39
			3	.83	.23	.34	.38	.34	.61
			4	.81	.08	0	.06	.09	0
		2	1	.77	.01	0	0	0	0
			2	.73	.01	.07	.005	.10	.09
			3	.71	.82	.58	.95	.84	.89
			4	.67	.14	.33	.04	.04	.02
			5	.63	.02	.02	.005	.02	0
		3	1	.62	0	0	0	0	0
			2	.60	.28	0	.13	.06	.01
			3	.58	.67	.92	.85	.84	.74
			4	.53	.05	.06	.02	.08	.17
			5	.50	0	.02	0	.02	.08

continued

Table 1. Continued.

Enzyme	Abbrev.	Locus	Allozyme	Relative Mobility	Species: occidentalis	retiniana	fumiferana	biennis	lambertiana
		5	1	.34	0	0	.05	.02	0
			2	.28	.01	0	.06	.01	.03
			3	.24	.03	.02	.46	.09	0
			4	.21	0	.10	.07	.04	.08
			5	.18	.07	.17	.12	.04	.34
			6	.06	.86	.69	.23	.75	.46
			7	.02	.01	.02	.02	.04	.09
		6	1	-.20	0	0	0	.01	0
			2	-.12	.07	.04	.02	.04	.18
			3	-.06	.93	.96	.98	.95	.82
a-Glycerophosphate dehydrogenase	AGPDH	1	1	.28	1.0	1.0	1.0	1.0	1.0
Isocitrate dehydrogenase	IDH	1	1	.38	0	0	0	0	0
			2	.31	1.0	.93	.98	1.0	1.0
			3	.24	0	.07	.02	0	0
Lactate dehydrogenase	LDH	1	1	.32	0	0	.03	0	0
			2	.24	.97	1.0	.97	.97	1.0
			3	.16	.03	0	0	.03	0
Leucyl amino-peptidase	LAP	2	1	.53	.27	.59	.72	.12	.10
			2	.50	.73	.41	.27	.88	.90
			3	.46	0	0	.01	0	0
		3	1	.36	0	.12	.04	.01	.01
			2	.34	1.0	.83	.96	.99	.99
			3	.31	0	.05	0	0	0
Malic enzyme	ME	1	1	.29	0	.08	.02	0	.20
			2	.22	.92	.88	.94	.98	.79
			3	.18	.04	.02	.02	.02	.10
			4	.16	.04	.02	.02	0	0

continued

Table 1. Continued.

Enzyme	Abbrev.	Locus	Allozyme	Relative Mobility	Species: *occidentalis*	*retiniana*	*fumiferana*	*biennis*	*lambertiana*
Malate dehydrogenase	MDH	2	1	-.30	0	0	0	0	.10
				-.24	1.0	1.0	1.0	1.0	.90
6-Phosphoglucose dehydrogenase	6PGDH	1	1	.14	1.0	1.0	1.0	1.0	-
Phosphoglucose isomerase	PGI	1	1	.16	.04	.01	.04	.09	-
			2	.12	.93	.98	.90	.83	-
			3	.04	.02	.01	.03	.05	-
			4	.01	.01	0	.03	.03	-
Xanthine dehydrogenase	XDH	1	1	.23	1.0	1.0	1.0	1.0	1.0

Table 2. Nei's genetic identity (below diagonal) and genetic distance (above diagonal) values for pairwise comparison of 5 *Choristoneura* species.

	occidentalis	*retiniana*	*fumiferana*	*biennis*	*lambertiana*
occidentalis	---	.020	.087	.007	.094
retiniana	.981	---	.075	.025	.113
fumiferana	.917	.928	---	.084	.173
biennis	.993	.975	.919	---	.077
lambertiana	.910	.893	.841	.926	---

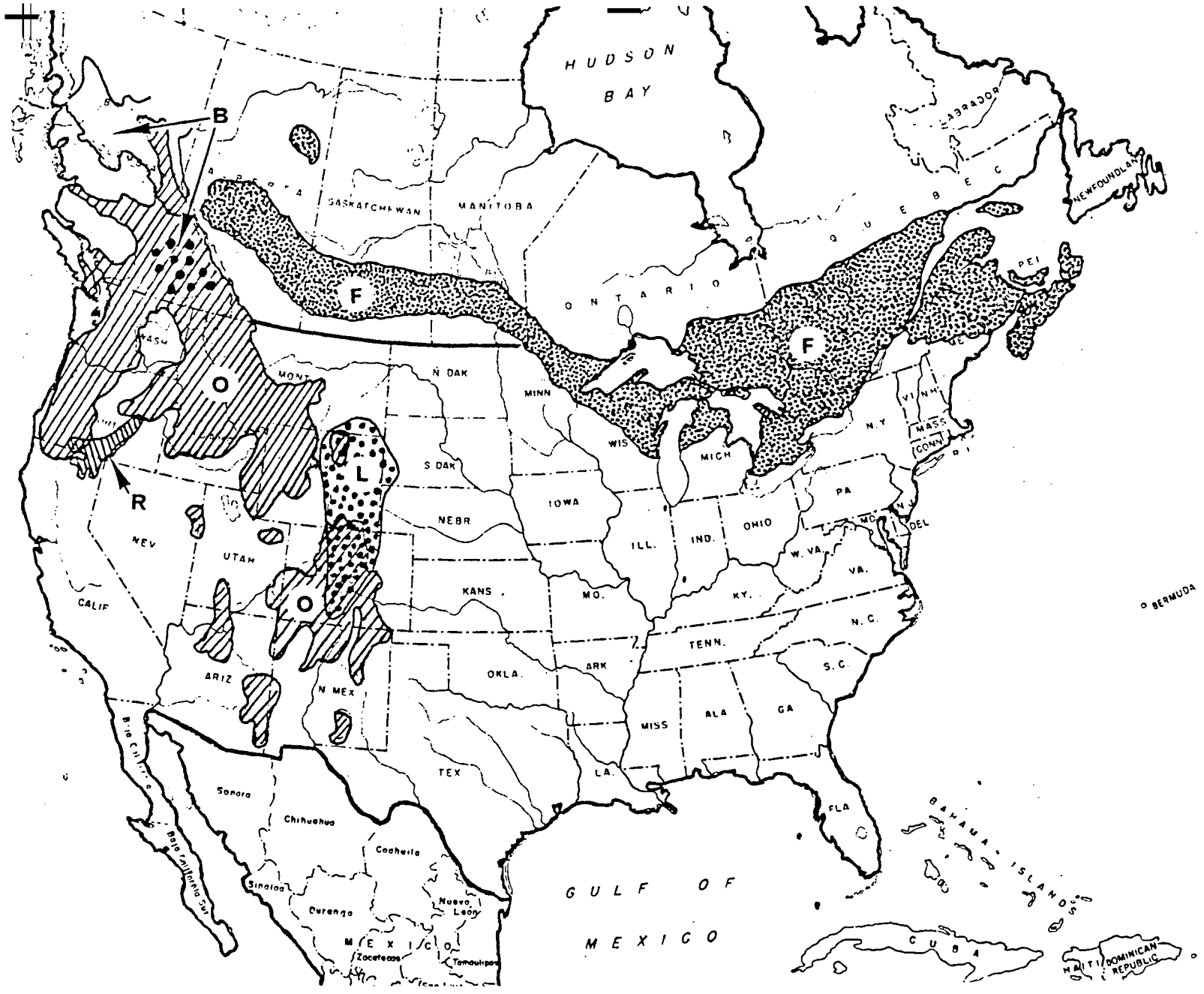

Figure 1. Approximate distribution of 5 Choristoneura species in North America. F = C. fumiferana, O = C. occidentalis, B = C. biennis, R = C. retiniana, and L = C. lambertiana ponderosana.

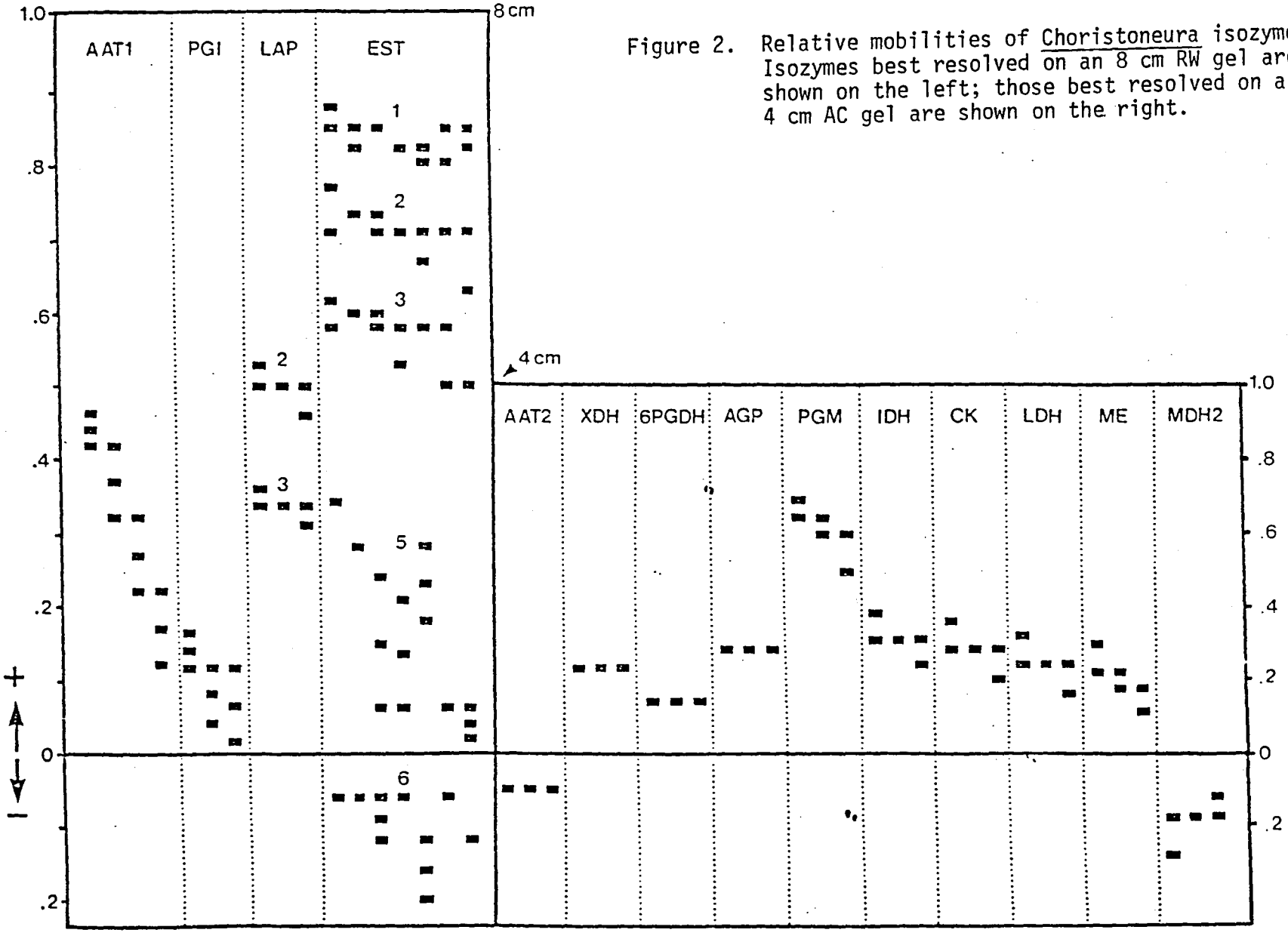

Figure 2. Relative mobilities of Choristoneura isozymes. Isozymes best resolved on an 8 cm RW gel are shown on the left; those best resolved on a 4 cm AC gel are shown on the right.

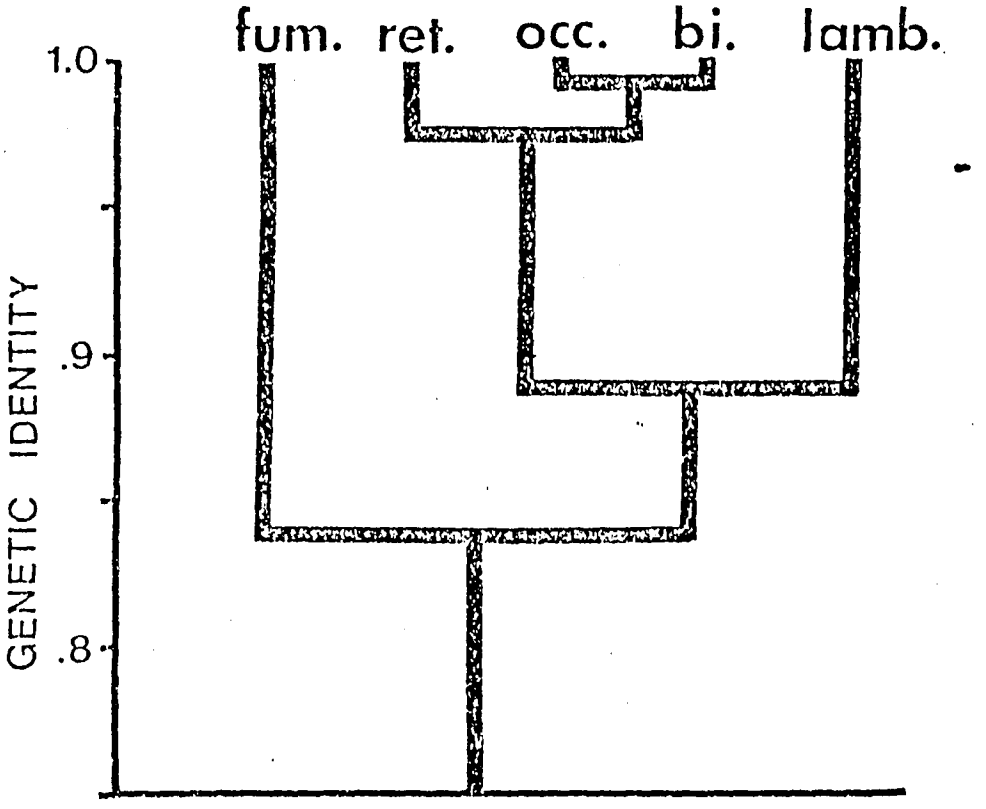

Figure 3. Dendrogram showing genetic relationships among Choristoneura species.

5. DISCUSSION AND CONCLUSIONS

The ecological division between the spruce/fir-feeding Fumiferana complex and the pine-feeding Lambertiana complex is considered quite distinct (Stehr 1967, Powell 1980). There are no confirmed records of oviposition by either of the 2 groups on reciprocal hosts leading to successful establishment of larvae. I therefore expected C. lambertiana ponderosana (the only pine-feeder of the 5 species compared) to be most reproductively isolated. However, these genetic data indicate that C. fumiferana is most distantly related to all other groups. This disparity could reflect the large geographic distance separating the C. fumiferana sample, taken in Maine, from the other groups. However, C. fumiferana from the Great Lakes area (Stock and Robertson 1980) were genetically very similar to the Maine population. Also, phenetic studies based on 11 morphological and 3 biological characters placed C. lambertiana subretiniana Obr., a pine-feeder sympatric with C. retiniana in northern California, between C. retiniana and C. carnana (Barnes and Busck), a Douglas-fir-feeder also found in California[2].

Problems associated with determining taxonomic relationships among the western spruce/fir-feeding species reflect the geographic complexity of western forest regions with their intermingling of tree species and their small-scale patterning of pure stands (Stehr 1967). Although host preferences generally differ among C. occidentalis, C. biennis, and C. retiniana, sympatry occurs in some areas of their distribution. The montane habitat of C. occidentalis

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[2] Pers. communication from J. DeBenedictis, Division of Entomology and Parasitology, University of California, Berkely, California 94720.

is, at upper elevations, frequently in contact with the subalpine spruce/fir habitat of C. biennis, thus permitting contact between the 2 species (Shepherd and Dolph 1979). However, differences in life cycles between C. occidentalis and C. biennis effect temporal isolation even where their ranges overlap. The ranges of C. occidentalis and C. retiniana also overlap and morphotypes representative of these species may be found together in the same stands[3]. In south-central Oregon, populations containing larvae and adults showing a morphological and biological gradation between C. occidentalis and C. retiniana occur (Powell 1980).

These relationships are reflected in the high levels of genetic similarity noted between C. occidentalis, C. biennis, and C. retiniana, and their relatively greater genetic distance from the western pine-feeding C. lambertiana and the eastern population of C. fumiferana. Genetic identity values calculated among the 5 species ranged from .99-.94. In other organisms, including many insect groups, values in this range are more commonly found between conspecific populations than between different species (Ayala 1975). Furthermore, fixation for different allozymes at a locus, an important indicator of reproductive and genetic divergence, was seen at no loci among the Choristoneura species studied here. These genetic data therefore suggest that reproductive isolation among at least some of these groups is not well established. This conclusion supports results of other investigations of biosystematic relationships within the genus Choristoneura. For example, in cross-mating

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[3] Pers. communication from W. Waters, Division of Entomology and Parasitology, University of California, Berkeley, California 94720.

experiments, Campbell (1967) found no evidence that C. occidentalis and C. biennis were reproductively isolated from each other. Sanders et al. (1977) found the Choristoneura species a convenient group for studies on the effects of hybridization on sex pheromone response because they readily hybridize in the laboratory.

Key traits used to distinguish the 3 western species are closely related to ecological and geographic factors to which these groups are exposed. For example, a key feature distinguishing C. occidentalis from C. biennis is the occurrence of a 2nd diapause in C. biennis. However, a 2nd diapause is regularly found in some individuals of other Choristoneura species and is readily elicited in most members of these species by a short photoperiod following completion of the 1st diapause (Harvey 1967). While only in C. biennis is the 2nd diapause common under natural conditions, Campbell (1953) found significant increases in the incidence of such a diapause in C. occidentalis reared at higher elevations. Thus, while the 2nd diapause in C. biennis is highly fixed genetically, it appears to be a recent adaptation to specific environmental conditions (e.g., the shortened growing season over its distribution as compared to that of C. occidentalis).

Measurements of head and prothoracic shield pigmentation of last-instar Choristoneura larvae, in addition to characters of pupal cuticle and hemolymph color, have been used to support taxonomic differentiation within this genus (Harvey and Stehr 1967). However, with respect to cuticle color, a north/south grouping of species has been noted; northern C. biennis, C. orae, and C. fumiferana are darker than more southern groups such as C. occidentalis, C. retiniana, and C. lambertiana. In many cases, such geographic differences in insect pigmentation have been attributed primarily to temperature effects, cooler temperatures favoring the deposition of melanic pigmentation (Rappaport 1969,

Stock 1980). Thus, while descriptions of color variation in Choristoneura immatures may have utility in identification of species locally, it is possible that the very dark pigmentation observed in C. biennis may be linked to low temperatures found at high elevations and the more northerly distribution of this species.

Based on genetic data and this brief review of the ecology and distribution of 5 Choristoneura species, we agree the Powell (1980) that definition of some of the Choristoneura species as reproductively isolated entities of comparable rank or genetic integrity is, at this point, artificial. Nevertheless, current Choristoneura species designation do have practical utility as markers of population types with specific host/habitat characteristics and are relevant to the development of pest management strategies for these insects.

6. WORK REMAINING ON STUDY

Objectives outlined in proposal (5 February 1979) have been met. Part 2 of objective 3 (identification of isozyme patterns which can be used to distinguish species) has only been achieved for C. fumiferana vs. the western species, however, due to the apparent lack of reproductive isolation among groups. Gene frequency differences between species and within C. occidentalis therefore appear most applicable to studies of budworm dispersal and outbreak spread and identification of environmental factors affecting gene flow, rather than for species identification.

7. COOPERATION AND COORDINATION

We thank R. F. Shepherd and J. A. McLean for collections of C. biennis, W. E. Waters and W. J. Volney for C. retiniana, R. E. Stevens for C. lambertiana ponderosana, and D. E. Leonard for C. fumiferana. We thank W. E. Waters, J. DeBenedictis, J. A. Powell, G. T. Harvey, and R. E. Stevens for reviewing results of this study and the contents of this report.

8. PROBLEMS ENCOUNTERED

None to speak of.

8b. SUGGESTIONS FOR FOLLOW-UP RESEARCH

The main area where follow-up work on the genetics of Choristoneura species would be helpful is in further examination of genetic differences among populations from east to west through Canada. Because C. fumiferana appears quite distinct from the western species, but has a distribution which meets that of some of the western species in western Canada, such studies would provide further information on levels of reproductive isolation among groups.

9. MANUSCRIPTS PREPARED

A manuscript entitled "Genetic Relationships Among Representative Populations of Five Choristoneura Species (Lepidoptera:Tortricidae): C. occidentalis, C. retiniana, C. biennis, C. lambertiana, and C. fumiferana" is in the final stages of preparation for submission to The Canadian Entomol-

ogist. The report presented here represents the majority of the contents of that paper but there will be more modification to the Discussion and to the Figures, especially Figure 1 (the map of species distributions). A copy of the manuscript will be forwarded to CANUSA-West when it is ready for submission to Can. Ent.

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